see original article on page 412

Microparticles and microRNAs of endothelial progenitor cells ameliorate acute kidney injury

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Horizontal information transfer between cells via microparticles is a newly identified communication system. MicroRNAs regulate gene expression and are detected in microparticles. Cantaluppi *et al.* suggest that microparticles derived from circulating angiogenic cells—'endothelial progenitor cells' (EPCs)—harbor endothelial-protective miRNAs such as miR-126 and that delivery of EPC-derived microparticles during acute kidney ischemia–reperfusion in rats ameliorates kidney dysfunction and damage. We highlight the importance, potential future impact, and limitations of this study.

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Most if not all cell types of diverse organisms release membranous nano-sized vesicles that harbor RNA, proteins, and lipids. These microparticles (MPs) include microvesicles (100 nm to 1 μm) generated by surface shedding of plasma membrane containing cytoplasm; and exosomes (40–100 nm) originating from the membrane of endosomal multivesicular bodies (MVBs) and released into the extracellular environment during exocytosis upon fusion with the plasma membrane. The function of these extracellular vesicles is just beginning to become apparent. They play a role in cell–cell communication and may have pathological functions in immune processes and cancer progression (Figure 1).

In kidney research, urinary MPs are being actively investigated as biomarkers and mediators of extracellular communication between renal epithelial cells.[1] Currently, only a few studies have examined the impact of MPs derived from extrarenal cells on kidney injury. MPs originating from mesenchymal stem and endothelial progenitor cells were found to convey signals that ameliorate tubular injury. For endothelial cell-derived MPs, horizontal transfer of mRNA and activation of an angiogenic program in the recipient endothelial cells has been suggested as the underlying mechanism.[2] MicroRNAs (miRNAs), small regulatory RNA molecules, are actively secreted packaged in MPs and/or bound to proteins from different cell types, including mast cells and embryonic stem cells.[3] Cantallupi *et al.*[4] (this issue) show that MPs from endothelial progenitor cells (EPCs) isolated from peripheral blood mononuclear cells of healthy human donors protect against acute and long-term consequences of ischemia–reperfusion injury in rats, if injected in the tail vein immediately after ischemic injury. MPs derived from fibroblasts or from EPCs treated with Dicer small interfering RNA or miR-126 and miR-296 inhibitors, or preincubated with RNase, failed to ameliorate ischemia–reperfusion injury. In addition, hypoxia-induced uptake of MPs diminishes apoptosis and promotes proangiogenic and antiapoptotic gene expression in cultured primary tubular endothelial and epithelial cells, in a manner at least partially dependent on miR-126 and miR-296.

These findings support previous work by the authors and other groups on the role of progenitor cells in kidney injury but also point out gaps in our understanding of MPs and their cargo: What is the difference between vesicles that ameliorate injury (MPs derived from EPCs) and those that have less or no effect (MPs from fibroblasts)? In this respect it needs to be emphasized that the term 'EPC' is indeed controversial, as it may include multiple cell types with different functions. EPCs circulate in the bloodstream and contribute mainly by paracrine actions to formation of new blood vessels, endothelial repair, and vascular homeostasis.[5] Impairment of EPCs is related to endothelial dysfunction and adverse clinical outcome.[6] EPCs are obtained by an adhesion-related isolation method and are defined by expression of the endothelial lineage markers, such as CD31, KDR (VEGFR-2), VE-cadherin, and von Willebrand factor. EPCs also show certain endothelial properties, such as migration toward proangiogenic factors. However, these early EPCs are also referred to as circulating angiogenic cells, monocytic EPCs, early-outgrowth EPCs, or angiogenic progenitor cells.[5] The cell type used by many groups and investigated by Camussi's group (Camussi *et al.*[2,3] and Cantaluppi *et al.*[4]) can also be described as circulating angiogenic cells. Future research will have to determine whether the findings of Camussi's group with regard to the observed beneficial effects and miRNA content are generally comparable among different 'EPC' subtypes identified so far.

The current paper by Cantaluppi *et al.*[4] attributes the protective effect to miR-126 and miR-296, which are detected in the microvesicles by reverse transcription PCR. Mouse knockout of miR-126 was found to be the cause of a severe vascular phenotype initially ascribed to its host gene, *Egfl7*.[7] Like its host gene, miR-126 is primarily expressed in endothelium. In a large small-RNA sequencing database, miR-126 composed up to 10–15% of the total miRNA content in tissues with a dominant endothelial component (angiosarcoma,

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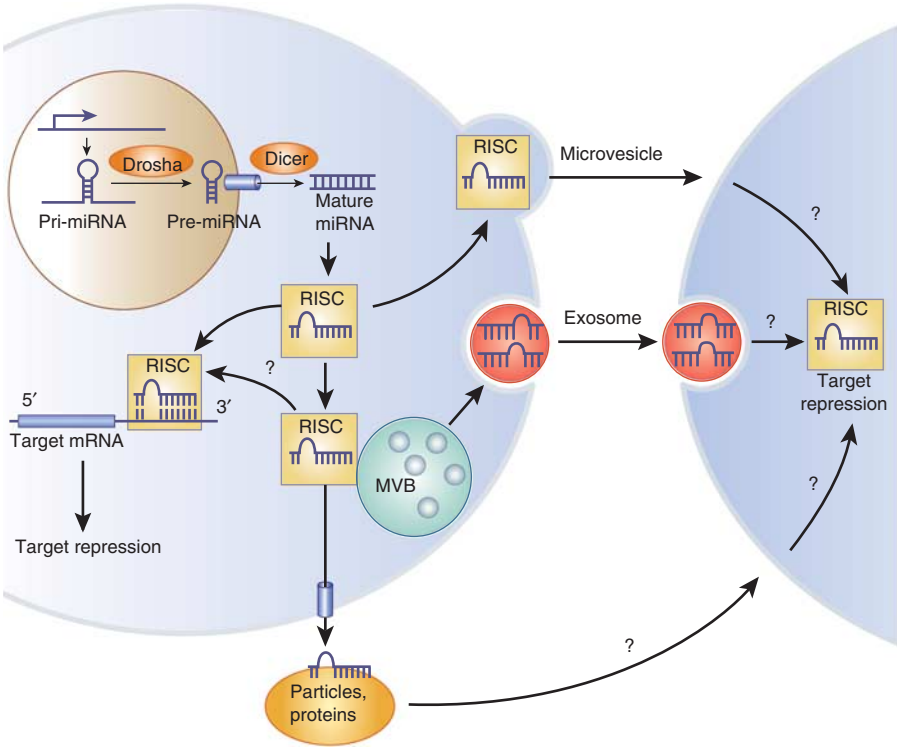

Figure 1 | MicroRNA biogenesis and transport. MicroRNAs (miRNAs) are transcribed from miRNA genes that are similar to protein-coding genes into primary miRNAs (pri-miRNAs; several hundreds of nucleotides long, harboring 5′ cap and 3′ poly-A tail), further processed into pre-miRNAs (~70 nucleotides long with a characteristic hairpin loop) by the RNase III enzyme Drosha in the nucleus, and shuttled into the cytoplasm through Exportin 5, where the RNase III enzyme Dicer generates mature miRNAs and passenger strand. Mature miRNAs are then incorporated into the RNA-induced silencing complex (RISC), where target mRNAs are either degraded or translationally repressed through partial sequence complementation. In the extracellular space, miRNAs have been detected in microvesicles and exosomes, and bound to extracellular particles and proteins. The RISC has been found to be associated with the membranes of multivesicular bodies (MVBs) derived from the endosome and in microvesicles; it is probably also in exosomes. The mechanisms underlying uptake of miRNAs into vesicles and the release from the vesicles in target cells are largely unknown.

microdissected glomeruli, heart), with levels of about 3–5% noted in CD34$^+$ lymphocytes, peripheral blood mononuclear cells, and fetal lung (T. Tuschl, personal communication, Laboratory of RNA Molecular Biology, Howard Hughes Medical Institute, Rockefeller University, New York, NY). As EPCs frequently can take up platelets and thus also their molecular content, the real source of certain miRNAs detected in EPCs remains to be determined. MiR-126 and many of its targets are highly expressed in endothelial cells, and both miR-126 and miR-296 have been implicated in angiogenesis[8] (although expression in the tissue database was not nearly as high for miR-296, and no enrichment was noted in samples containing endothelium). The notion that miRNAs are also transferred to other cell types urges us now to identify also cell type-specific targets of transferred miRNAs, especially if they are normally not expressed in this cell type, which is the case for tubular epithelial cells and miR-126 (I.Z.B.-D., unpublished observations). Thus, the targets of miR-126 in diverse kidney cells remain to be determined.

MiRNAs might be enriched in MPs, and the machinery required for miRNA function has recently been linked to multivesicular bodies (MVBs). MiRNAs require the RNA-induced silencing complex (RISC) for downregulation of expression of target genes, but formation and turnover of the RISC itself are tightly regulated and require endosomal membranes. MVBs associate with RISC and GW bodies, and the blocking of MVB formation results in impaired silencing of mRNA by miRNAs, indicating that active RISC is physically and functionally coupled to MVBs.[9] It remains to be determined whether this association results in enrichment of RISC-associated small RNAs in exosomes, which originate from MVBs, and in regulation of miRNA content in MPs in physiology and disease.

Notwithstanding the above discussion, it cannot be ruled out that the cargo RNA mediator of the protective effect of MPs is not the miRNA but an indirect mRNA target. For this to happen, the putative transcript would have to be upregulated by miR-126; that is, miR-126 would target a repressor of this mRNA. In fact, the latter mechanism may be favored on the basis of stoichiometric considerations. Assuming the total take-up of MPs by the cell amounts to 1:1000 of its volume (probably an overestimate), the delivered miRNA, even if enriched in the vesicle, is much diluted compared with its concentration in the parent cell. Since miRNAs perform their function on a 1:1 basis with their targets (and must compete with 'native' miRNA in RISC loading), significant regulation of a target gene is implausible in most cases. On the other hand, a newly introduced mRNA is more likely to cause a switch, as it can be used to generate many protein molecules, departing from the 1:1 relationship. Additionally, mRNAs are also more susceptible to ribonuclease digestion, which is consistent with the findings of Cantaluppi *et al.*[4] that RNase treatment abolishes the effect of MPs.

The findings reported by Cantaluppi *et al.*[4] suggest that MPs from unchallenged EPCs protect from ischemia–reperfusion injury. It can be speculated whether circulating MPs constitute an additional physiological mechanism to counter endothelial damage that may be altered in disease states. Furthermore, the authors show that dye-labeled MPs were detected not only in endothelial but also in tubular epithelial cells 2 hours after tail vein injection and that MPs have measurable effects in cultured tubular epithelial cells. As MPs appear to be too large to enter the tubular lumen through the glomerular filtration barrier to reach the tubular epithelial cells via the urinary space, MPs would need to migrate from the blood through the basal membrane to the tubular epithelium.

Current knowledge supports further exploration of the complex interplay between the microRNA machinery and endosomal vesicles, as well as the mechanisms of cargo loading into microvesicles and exosomes, the recognition of target cells by MPs, and the alteration of gene and protein expression in target cells by cargo of MPs (Figure 1). Better understanding of the biology of MPs and their content is important before genetically altered vesicles can be considered as potential novel therapeutic carriers.

DISCLOSURE

The authors declared no competing interests.

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see original article on page 428

Intrarenal urothelial transformation after obstruction: a novel metaplasia

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Obstructive nephropathy is a major cause of acute renal failure and end-stage renal disease. We discuss the new findings of Girshovich *et al.*, who show that transformation of intrarenal urothelium into a bladder-like urothelium depends on the activation of the FGF7–FGFR2 signaling pathway following acute ureteral obstruction. A possible link between hypoxia-inducible factor 1α and the FGF–FGFR signaling pathway is suggested.

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Obstructive nephropathy is one of the common causes of acute renal failure and end-stage renal disease. Many animal models have been developed to study the renal consequences of ureteral obstruction in the events of acute, chronic, or congenital ureteropelvic junction obstruction.[1] Most studies on renal parenchyma alterations have focused on glomerular changes, tubulointerstitial injury, and fibrosis.[2,3] Instead, Girshovich *et al.*[4] (this issue) turn their attention to a small group of cells called the intrarenal urothelium that resides near the fornices (corticomedullary junction).

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The intrarenal urothelium is morphologically and phenotypically distinct from the bladder urothelium. The renal urothelium, near the fornices, is composed of one- or two-layered epithelium, whereas the bladder epithelium is a multilayered epithelium consisting of basal cells, intermediate cells, and superficial umbrella cells. The apical surface of a bladder urothelium contains urothelial plaques that contribute to a tight permeability barrier, thus preventing rupture during bladder distension and the influx of urine from the bladder lumen. The urothelial plaques contain four major integral membrane proteins, uroplakins Ia, Ib, II, and III.[5] The renal urothelium near the fornices, in contrast, showed much weaker uroplakin immunostaining compared with typical umbrella cells of the bladder urothelium.[6] Although the bladder urothelium is constantly subjected to pressure changes, no significant stretching occurs in the intrarenal urothelium under physiological conditions. In the event of hydronephrosis secondary to ureteral obstruction, the intrarenal urothelium becomes the first barrier subjected to pressure-induced stretching and deformation.

Using an adult mouse unilateral ureteral obstruction (UUO) model, Girshovich *et al.*[4] designed a series of experiments to examine the morphological and phenotypical alterations of intrarenal urothelium subjected to increased intrapelvic pressure. They were able to demonstrate that the intrarenal urothelium differentiated into a 'bladder-like' urothelium following UUO. This bladder-like urothelium was characterized by a multilayered epithelium with strong expression of uroplakins Ib, II, and III. These findings were further substantiated by electron microscopy examination, in which the transformed renal urothelium within the obstructed kidney showed abundant uroplakin-containing vesicles and thickened asymmetric unit membrane covering the entire apical surface, suggesting a much more efficient barrier to water and solutes compared with normal renal urothelium.

The authors also made several very interesting observations. There was a significant increase in the proliferation index